Design and Engineering of Nanopores with Emergent Functions

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interaction partners. How difficult, then, is it for a protein to discriminate its correct interaction partner(s) from the possibly large set of other proteins it may encounter in the cell?
An important ingredient of recognition is shape complementarity. The ensemble of protein shapes should be constrained by the need for maintaining functional interactions while avoiding spurious ones.
To address this aspect of protein recognition, we consider the ensemble of proteins in terms of their three-dimensional shapes, more precisely in terms of their solvent-excluded surfaces. We take into account the high-resolution structures from E.coli non-DNA-binding cytoplasmic proteins that can be retrieved from the Protein Data Bank. We aim to characterize the statistical properties of the protein surfaces at two levels: First, we study the intrinsic dimensionality at the level of the ensemble of the surface objects. Second, at the level of the individual surfaces, we determine the scale of shape variation. We further discuss how the dimensionality of the space of protein surfaces is linked to the statistical properties of individual protein surfaces.

2642-Pos Board B19
A Novel in Silico 4D Geometrical Measure of the Active Site Correlates with the Enzymatic Activity of HCV NS3 Protease; Implications in Catalysis and Drug Design
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We have previously developed and improved on a 4D computational methodology, based on 3D structural modeling coupled with molecular dynamics simulations, to analyze, pan-genotypic, the active sites of the NS3 proteases of HCV, in relation to their catalytic activity and drug susceptibility. The 4D analysis of the interactions between the catalytic triad residues (His57, Asp81, and Ser139) yielded a divergent, gradual and genotype- dependent, 4D conformational instability measures, which correlate well the with known altered catalytic activities. Here, we present the correlation of our 4D instability measures to known intra-genotypic alterations in NS3 protease activity, due to sequence variations in the NS4A cofactor. The correlation is qualitatively evident, which further validates our methodology, paving the way to building an accurate quantitative metric to predict protease activity in Silico. In addition, the results suggest a plausible ''information'' pathway from the activation subunit (the NS4 cofactor binding site) to the catalytic subunit (the catalytic triad region). The results strongly suggest that the structural dynamics behaviour, more than the rigid structure, is related to the altered catalytic activity and possibly drug susceptibility seen in HCV NS3 proteases.

2643-Pos Board B20
Tensile Mechanics of Coiled Coil Protein Structures
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A coiled-coil protein structure consists of two or more interacting α-helical strands that together form a supercoil structure. Coiled-coil structures entail unique mechanical properties that are critical to the function and integrity of various motor proteins, cytoskeletal filaments and extra-cellular matrix proteins. Here we present a thermodynamic model to predict the mechanical properties of a given coiled-coil structural motif. Within the proposed model we identify and quantify various energetic and entropic effects, responsible for dimerization of two helical polypeptides into a coiled coil structure. We determine our model parameters by examining a large body of solved protein structures that contain coiled coil motifs. This would allow us to develop a thermodynamic model for predicting the propensity of given amino acid sequence to form a coiled coil structure. Further incorporation of the above model into our previously developed α-helix tensile mechanics model would enable us to predict the structural response of a given coiled coil motif to a tensile stress.

2644-Pos Board B21
Design and Engineering of Nanopores with Emergent Functions
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Engineering new functions into existing assemblies is a first step to build artificial biological systems. Further, the emergence of an enzymatic function can reveal functional insights and allows engineering biological systems with enhanced properties. Biological nanopores with known structure are ideal building elements for this task because they have a robust assembly that allows precise engineering. For example, we recently incorporated DNA molecules atop a ClyA nanopore to build artificial transporters that are able to selectively shuttle DNA or proteins across a lipid membrane. Nanopores have additional advantages, as the ionic flux through the nanopore can be exploited to recognize molecules or study biological and chemical processes at the single-molecule level.
Here we engineered an alpha hemolysin nanopore to operate as GroES, a co-chaperonin that in complex with GroEL forms a two-stroke protein-folding nanomachine. The new function was introduced bottom-up by adding modular elements into an unrelated globular structure, suggesting a possible path during GroEL-GroES evolution in which the co-chaperonin function appeared sequentially by incorporating hydrophobic polypeptide loops into pre-existing protein assemblies. The binding of GroEL to individual GroES-nanopores prompted large changes to the unitary nanopore current, most likely reflecting the allosteric transitions of the chaperonin nanomachine. In the presence of unfolded proteins the pattern of current transitions changed, suggesting a possible mechanism in which the free energy of ATP binding and hydrolysis is expended only when substrate proteins are occupied.

2645-Pos Board B22
On the Combination of Restraint-Driven Docking of Flexible Peptides to Ion Channels - Lessons Learnt from the Complex Formed by the Spider Venom PcTx1 and the Acid Sensing Ion Channel1
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Venom peptides that bind to ion channels have attracted much interest as potential lead molecules for pharmaceutical development. However, experiments to determine the structure of large peptide-channel complexes are challenging. Instead, structural models are often derived by combining experimental information with restraint-driven docking. The question is how reliable are such models and what is the most effective use of available experimental data. Also, structures obtained from docking are often assessed using geometric criteria, which might of limited use to an experimentalist aiming to identify the interactions at the binding interface for lead optimization. Using the complex formed by the venom peptide PcTx1 and the acid sensing ion channel 1 as a case study, we to examined the likelihood of using restraint-driven docking to find a structure that can be used to identify interfacial residues and to predict specific pairwise peptide-channel interactions.
For this, we have analysed over 240'000 docked structures of the PcTx1-ASIC1a complex, produced using HADDOCK [1]. This showed that when using information on residues involved in binding for both the peptide and the channel, there is a ~60% chance of predicting a structure with an interface-RMSD < 4Å compared to the PcTx1-ASIC1a co-crystal structure. The likelihood of correctly identifying specific pairwise peptide-channel interactions was ~30%. Furthermore, we will show how the effect of variability between docking runs should be considered when carrying out restraint-driven docking of peptide-channel complexes. Finally, we reflect on the limitations of relying on geometric criteria such as RMSD to assess the accuracy of docking procedures for lead optimization.
1. Dominguez, C.; Boelens, R.; Bonvin, A. M., J. Am. Chem. Soc. 2003.

2646-Pos Board B23
***de novo* Designed Peptides Inhibit the Cytokines Binding to their Receptors from Molecular Simulations and in Vitro Experiments**
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Sepsis, blood poisoning, is a systemic inflammatory response syndrome (SIRS) with high incidence and mortality. An overwhelming systemic response brought about by the release of various inflammatory mediators can lead to shock, multiple organ damage and death. Up to date, several therapeutic agents have been tested in clinical trails of sepsis, but the significant survival advantage is still limited. Some proinflammatory cytokines, such as TNF-α released from LPS-activated macrophages, IL-6, and IL-1β, also have much influence on the septic shock and ;multiple organ dysfunction syndrome (MODS).
The aim of this study is to design new peptides to inhibit the cytokines, such as TNF-α, IL-6, and IL-1β, binding to reduce their concentrations in the blood of patients with severe sepsis and to interrupt the initial cytokine cascade. The peptides determined from the binding sites of resolved cytokine-receptor complexes structures were firstly re-docked to validate the selected peptide-cytokine binding and peptides property. These peptides were then combined as a *de novo* peptide with about 20 amino acids to search for more preferable binding affinity. More than 200 ns molecular dynamics simulations were